Poster 175
F1-2156

50th Annual ICAAC
12-15 September, 2010
Boston, MA

TP-271, a Novel Oral Fluorocycline for Community-Acquired Respiratory and Biothreat Pathogens

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Revised Abstract

Background: TP-271 is a fully synthetic fluorocycline, with potent *in vitro* and *in vivo* antibacterial activity against MDR, including tetracycline-resistant, respiratory pathogens.

Method: *In* vitro susceptibility testing against panels of recent, diverse MDR clinical isolates and genetic diversity sets of five biodefense select agents was done by CLSI guidelines; tetracycline-specific resistance genes were detected by PCR. Efficacy was tested in cytoxan-treated BALB/c mice infected intranasally (IN) with *Streptococcus pneumoniae tet*(M) or MRSA *tet*(M), dosed at 2 and 12 hrs post-infection, and colony forming units (CFUs) in lung were quantified at 24 hrs post-initial dose. Efficacy was tested in immunocompetent CD-1 mice IN infected with *S. pneumoniae*, with compound dosed at 5, 24, and 36 hrs post-infection and lung CFUs quantified at 48 hrs. Efficacy against *H. influenzae* was tested in Sprague-Dawley rats infected intratracheally and treated at 5, 24, and 48 hrs post-infection, with quantification of lung CFUs at 72 hrs.

Results: TP-271 exhibited excellent *in vitro* activity against public health and biothreat respiratory pathogens (Table 1 and 2). In neutropenic lung models with MRSA and *S. pneumoniae*, TP-271 produced 2-3 log reductions in lung CFUs when dosed orally (50 and 30 mg/kg, respectively), and 3-4.5 log reductions dosed IV at 10 mg/kg (Fig 2A & B). In an immunocompetent lung infection model with *S. pneumoniae,* TP-271 provided a 4.8 log reduction in lung CFUs orally at 30 mg/kg (Fig. 2C). TP-271 was efficacious in a rat lung infection model with *H. influenzae*, producing 1.8 and 4.9 log reductions dosed orally at 100 mg/kg and IV at 25 mg/kg, respectively (Fig. 2D).

Conclusions: *In vitro* and *in vivo* efficacy of TP-271 supports its development for the treatment of community and biodefense respiratory indications.

Methods

Susceptibility testing. All minimal inhibitory concentration (MIC) assays were performed as per CLSI guidelines. Testing of public health pathogens was performed at Tetraphase Pharmaceuticals using recent clinical isolates obtained from Eurofins Medinet. Testing of biothreat agents was performed at United States Army Medical Research Institute for Infectious Diseases (USAMRIID).

Animal infection models. All animal infection models were done at ViviSource Laboratories, Waltham, Massachusetts.

Neutropenic *S. pneumoniae* Lung Model. BALB/c mice were challenged with tetracycline-resistant *tet*(M) *S. pneumoniae* strain SP160 (n=6 per group). The MICs for TP-271 and linezolid were ≤0.016 and 1 µg/mL, respectively. Mice were made neutropenic by pre-treatment with cyclophosphamide and infected with SP160 via intranasal administration. Mice were dosed orally with 30 mg/kg compound or IV with 10 mg/kg compound at 2 and 12 hours post-infection. At 24 hours following initiation of treatment, mice were euthanized and bacterial reduction in the lung was quantified by plating lung homogenates.

Immunocompetent *S. pneumoniae* Lung model. CD-1 mice were challenged with *S. pneumoniae* strain SP514 (n=6 per group). The MICs for TP-271 and linezolid were ≤0.008 and 0.5 µg/mL, respectively. Mice were infected with SP514 via intranasal administration. Mice were dosed orally with 30 mg/kg compound at 5, 24 and 36 hours post-infection. At 48 hours following initiation of treatment, mice were euthanized and bacterial reduction in the lung was quantified by plating lung homogenates.

Neutropenic MRSA Lung model. Neutropenic BALB/c were made neutropenic with cyclophosphomide and challenged with a tetracycline-resistant *tet*(M) MRSA strain SA191 (n=6 per group). The MICs for TP-271 and linezolid were 0.25 and 2 µg/mL, respectively. At 2 and 12 hours mice were dosed orally with 50 mg/kg compound and linezolid was dosed at 30 mg/kg. For IV administration, TP-271 and linezolid were dosed at 10 mg/kg at 2 and 12 hours. At 24 hours following initiation of treatment, mice were euthanized and bacterial reduction in the lung was quantified by plating lung homogenates.

Immunocompetent *H. influenzae* Lung model. TP-271 was tested in a rat lung infection challenged with *H. influenzae* HI551 (n=6 per group). The MICs for TP-271 and azithromycin were ≤0.016 and 0.5 µg/mL, respectively. At 5, 24, and 48 hours rats were dosed orally with 100 mg/kg TP-271 and azithromycin was dosed at 50 mg/kg. For IV administration, TP-271 was dosed at 25 mg/kg at 5, 24 and 48 hours. At 72 hours following initiation of treatment, rats were euthanized and bacterial reduction in the lung was quantified by plating lung homogenates.

Results

Figure 1. TP-271

Table 1. Determination of MIC_{50} and MIC_{90} values for respiratory pathogens

Organism	N	Antibiotic (µg/ml)												Resistant Phenotypes
		TP-271			Doxycycline			Linezolid			Levofloxacin			
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
S. aureus (MRSA)	32	0.031	0.13	≤0.016-1	0.13	8	0.063-16	2	4	2-4	16	>32	0.25->32	tet, tig, ery, levo
S. pneumoniae	19	≤0.016	≤0.016	≤0.016-≤0.016	1	8	≤0.016- 16	1	1	0.5-1	1	1	0.5-1	tet, pen, ery, clinda,
S. pyogenes	14	≤0.016	≤0.016	≤0.016-≤0.016	0.25	16	0.031-16	1	2	0.5-2	1	2	0.5-2	tet, ery, clinda,
H. influenzae	14	0.25	0.5	0.031-0.5	1	4	0.25-4	8	16	8-16	≤0.016	0.031	≤0.016–0.3	tet, amp
M. catarrhalis	14	≤0.016	≤0.016	≤0.016–0.063	0.25	8	≤0.016–8	4	8	0.5-8	0.063	0.063	0.031-0.25	tet, amp
A. baumannii	25	0.13	1	≤0.016-2	1	>32	0.031->32	nd	nd	nd	4	32	0.13->32	tet, carba, levo, gent

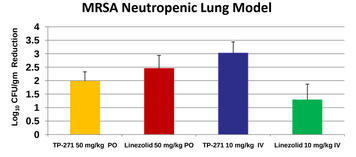
Table 2. Determination of MIC_{50} and MIC_{90} values for biothreat pathogens

Organism	N	Antibiotic (µg/ml)								
		TP-271			Doxycycline			Ciprofloxacin		
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Yersinia pestis	30	0.12	0.25	0.015-0.5	0.5	1	0.06-2	0.03	0.06	0.015-0.06
Bacillus anthracis	30	≤0.008	≤0.008	≤0.008- ≤0.008	≤0.008	≤0.008	≤0.008-0.015	0.12	0.25	0.03-0.25
Fransciscella tularensis	27	0.25	0.5	0.03-2	0.12	0.25	0.015-1	0.06	0.5	0.015-8
		TP-271			Doxycycline			Azithromycin		
Burkholderia mallei	30	0.06	0.12	≤0.008-1	0.03	0.12	≤0.008-1	1	2	0.25-2
		TP-271			Doxycycline			Ceftazidime		
Burkholderia pseudomallei	30	1	4	0.25-8	1	8	0.06-16	2	2	0.25-32

Results

Figure 2. TP-271 is active in murine respiratory infection models for *S. pneumoniae,* MRSA, and *H. influenzae*

A.



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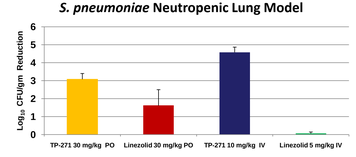
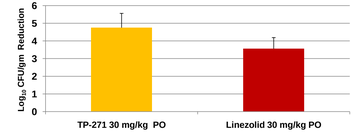
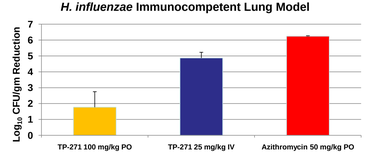


C.



D.



Conclusions

- TP-271, a unique fully synthetic tetracycline, exhibits excellent potency against key drug-resistant public health respiratory pathogens, including *S. pneumoniae,* MRSA, *H. influenzae, M. catarrhalis* and *Legionella pneumophila* (see poster F1-2159).
- Antibacterial activity is minimally affected by tetracycline-specific resistance mechanisms (see poster F1-2160).
- TP-271 also exhibits potent broad-spectrum activity against five important biothreat agents: *Y. pestis, B. anthracis, F. tularensis, B. mallei,* and *B. pseudomallei*
- TP-271 was active in murine respiratory models of infection with *S. pneumoniae, MRSA,* and *H. influenzae,* highlighting its potential for use in the treatment of serious respiratory infections caused by multidrug-resistant public health and biothreat pathogens.